

Amendments to the Claims

Claim 1 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising a polynucleotide sequence, the sequence comprising an exon marker sequence, the expression of which is obtained only upon integration of the polynucleotide construct into an actively transcribing genome region of the cell, wherein the marker sequence is flanked by a 5' splice acceptor sequence and a 3' splice donor sequence, wherein the exon marker sequence in a 5' to 3' direction contains:

two restriction enzyme recognition (RER) sites located at the 5' end of the marker exon, wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA upstream of the 5' end of the marker exon; and

two restriction enzyme recognition (RER) sites located at the 3' end of the marker exon, wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA downstream of the 3' end of the marker exon;

isolating mRNA from said cell;

reverse transcribing the isolated mRNA into double stranded cDNA;

subjecting the cDNA to digestion with a first one or more Type IIS restriction enzymes that ~~recognizes~~recognize ~~one of each of the~~ one of each of the Type IIS RER ~~sites~~site located at the 5' end

of the marker exon and one Type IIS RER site located at the and 3' ends-end of the
marker exon and cleavingso that the cDNA upstream of the 5' end of the marker exon and
downstream of the 3' end of the marker exon is cleaved, thereby producing a cDNA
fragment comprising the marker exon, and portions of upstream and downstream cellular
exon tags;

subjecting said cDNA fragment to a DNA polymerase and nucleotides to generate a blunt-ended
fragment;

self-ligating the cDNA fragment, thereby fusing the exon tags in opposing orientations;
amplifying a region of the cDNA fragment containing the exon tags in opposing orientations
thereby generating a linear DNA molecule containing the exon tags in opposing
orientations flanked by sequences corresponding to the marker exon 5' and 3' ends;
subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the
RER sites not previously recognized by the first Type IIS restriction ~~enzymes~~enzyme,
thereby generating a linear DNA fragment containing upstream and downstream exon
tags fused in an inverted conformation;
cloning the fragments comprising the tags in inverted conformation;
obtaining the nucleotide sequence of the cloned tags; and
comparing the individual sequence tags or pairs of sequence tags to a sequence database such
that the RNA transcript corresponding to the sequenced tags is identified.

Claim 2 (Original): The method of claim 1 further comprising ligating the amplified
fragments together to form a concatamer prior to cloning.

Claim 3 (Original): The method of claim 1, wherein the polynucleotide construct is contained within a vector.

Claim 4 (Original): The method of claim 3, wherein the vector is a viral vector.

Claim 5 (Original): The method of claim 4, wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector and an adeno-associated viral vector.

Claim 6 (Original): The method of claim 5, wherein the viral vector is a retroviral vector.

Claim 7 (Original): The method of claim 1, wherein the marker exon marker sequence encodes a fluorescent protein.

Claim 8 (Original): The method of claim 7, wherein the fluorescent protein is green fluorescent protein.

Claim 9 (Currently amended): The method of claim 8, wherein the fluorescent protein is detected and measured by ~~fluorescence-activated~~ flow cytometry.

Claim 10 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising a polynucleotide sequence, the sequence comprising an exon marker sequence, the expression of which is obtained only upon integration of the polynucleotide construct into an actively transcribing genome region of the cell, wherein the marker sequence is flanked by a 5' splice acceptor sequence and 3' splice donor sequence, wherein the exon marker sequence comprises in a 5' to 3' direction:

two restriction enzyme recognition (RER) sites located at the 5' end of the marker exon,

wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA upstream of the 5' end of the marker exon

two restriction enzyme recognition (RER) sites located at the 3' end of the marker exon,

wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the marker exon;

isolating mRNA from said cell;

reverse transcribing the isolated mRNA into single stranded cDNA using a primer of sequence complementary to the sequence of the marker exon;

extending the 3' end of the single stranded cDNA with a homopolymeric polydeoxynucleotide sequence using a single deoxynucleotide triphosphate and an enzyme terminal transferase;

synthesizing a second and complementary cDNA using a DNA polymerase and a primer

complementary to the homopolymeric sequence;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes one of the Type IIS RER ~~site~~site located at the 5' end of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon, and portions of upstream cellular exon tags;

ligating a linker to the cDNA fragment;

amplifying the linker and cDNA fragment with primers complementary to the marker exon and to the ligated linker;

subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction ~~enzymes~~enzyme;

cloning the fragments;

obtaining the nucleotide sequence of the cloned tags; and

comparing the individual sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 11 (Original): The method of claim 10 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claims 12-14 (Cancelled).

Claim 15 (Original): The method of claim 10, wherein the polynucleotide construct is contained within a vector.

Claims 16-17 (Cancelled).

Claim 18 (Original): The method of claim 15, wherein the vector is a viral vector.

Claim 19 (Original): The method of claim 18, wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector and an adeno-associated viral vector.

Claim 20 (Original): The method of claim 19, wherein the viral vector is a retroviral vector.

Claim 21 (Original): The method of claim 10, wherein the marker exon sequence encodes a fluorescent protein.

Claim 22 (Original): The method of claim 21, wherein the fluorescent protein is a green fluorescent protein.

Claim 23 (Currently amended): The method of claim 22, wherein the fluorescent protein is detected and measured by ~~fluorescence activated~~ flow cytometry.

Claim 24 (Currently amended): A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising a polynucleotide sequence, the polynucleotide sequence comprising an exon marker sequence, the expression of which is obtained only upon integration of the polynucleotide construct into an actively transcribing genome region of the cell, wherein the marker sequence is flanked at its 5' end by a splice acceptor sequence and at its 3' end by a splice donor sequence, wherein the exon marker sequence comprises in a 5' to 3' direction:

two restriction enzyme recognition (RER) sites located at the 5' end of the marker exon,

wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA upstream of the 5' end of the marker exon; and

two restriction enzyme recognition (RER) sites located at the 3' end of the marker exon,

wherein the RER sites are different from each other and, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the marker exon[.];

isolating mRNA from said cell;

reverse transcribing the isolated mRNA into single stranded cDNA;

synthesizing a second complementary strand of cDNA with a DNA polymerase enzyme and a

primer whose sequence corresponds to the sequence of the marker exon;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes one of the Type IIS RER

sites located at the 3' end of the marker exon and thereupon cleaves the cDNA

downstream of the 3' end of the marker exon, thereby producing a cDNA fragment

comprising the marker exon and portions of downstream cellular exon tags;
ligating a linker to the cDNA fragment comprising the marker exon fused to a downstream
flanking cellular exon tag;
amplifying the cellular exon tag with primers complementary to the marker exon and to the
ligated linker;
subjecting the amplification products to one or more restriction enzymes that recognize the RER
sites not previously recognized by the Type IIS restriction ~~enzymes~~enzyme used;
cloning the fragments;
obtaining the nucleotide sequence of the cloned fragments; and
comparing the individual sequence tags to a sequence database such that the RNA transcript
corresponding to the sequenced tags is identified.

Claim 25 (Original): The method of claim 24 further comprising ligating the amplified
fragments together to form a concatamer prior to cloning.

Claims 26-28 (Cancelled).

Claim 29 (Original): The method of claim 24, wherein the polynucleotide construct is
contained within a vector.

Claim 30 (Original): The method of claim 29, wherein the vector is a viral vector.

Claim 31 (Original): The method of claim 30, wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector and an adeno-associated viral vector.

Claim 32 (Original): The method of claim 31, wherein the viral vector is a retroviral vector.

Claim 33 (Original): The method of claim 24, wherein the exon marker sequence encodes a fluorescent protein.

Claim 34 (Original): The method of claim 33, wherein the fluorescent protein is green fluorescent protein.

Claim 35 (Currently amended): The method of claim 34, wherein the fluorescent protein is detected and measured by ~~fluorescence-activated~~ flow cytometry.

Claim 36 (Currently amended): A method for elucidating a RNA transcription profile in a cell comprising:
introducing into the cell a polynucleotide marker fragment, the expression of which is obtained only upon integration of the polynucleotide ~~construct~~ marker fragment into an actively transcribing genome region of the cell, wherein such polynucleotide comprises in a 5' to 3' direction:

two restriction enzyme recognition (RER) sites located at the 5' end of the fragment,

wherein the RER sites are different from each other and, wherein at least one of

the RER sites is recognized by a Type IIS restriction enzyme, and wherein this

RER site is oriented so that a Type IIS restriction enzyme will cut the DNA

upstream of the 5' end of the fragment; and

two restriction enzyme recognition (RER) sites located at the 3' end of the fragment,

wherein the RER sites are different from each other and, wherein at least one of

the RER sites is recognized by a Type IIS restriction enzyme, and wherein this

RER site is oriented so that a Type IIS restriction enzyme will cut DNA

downstream of the 3' end of the fragment;

isolating mRNA from said cell;

reverse transcribing isolated mRNA into double stranded cDNA;

subjecting the cDNA to digestion with ~~a first~~one or more Type IIS restriction-~~enzyme~~enzymes

that ~~recognizes~~recognize one ~~of each of the~~ Type IIS RER ~~sites~~site located at the 5' end of

the marker and one Type IIS RER site located at the~~and~~ 3' end of the marker fragment and

cleaving the cDNA upstream of the 5' end of the marker fragment and downstream of the

3' end of the marker fragment, thereby producing a cDNA fragment comprising the

marker fragment and portions of upstream and downstream cellular nucleotide ~~RNA~~

sequence tags;

self-ligating the cDNA fragment into a circular molecule, thereby fusing the two RNA tags in

opposing orientations;

amplifying a region of the cDNA fragment containing the tags in opposing orientations, thereby generating a linear DNA molecule containing two tags in opposing orientations flanked by sequences corresponding to the marker fragment 5' and 3' ends; subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the RER sites not previously recognized by the first Type IIS restriction ~~enzymes~~enzyme, thereby generating a linear DNA fragment containing two upstream and downstream tags fused in an inverted conformation; cloning the fragments comprising the tags in inverted conformation; obtaining the nucleotide sequence of the cloned fragments; and comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 37 (Original): The method of claim 36 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claim 38 (Currently amended): A method for elucidating a RNA transcription profile in a cell comprising:
introducing into a cell a polynucleotide marker fragment, the expression of which is obtained only upon integration of the polynucleotide ~~construct~~marker fragment into an actively transcribing genome region of the cell, wherein the polynucleotide marker fragment comprises in a 5' to 3' direction:
two restriction enzyme recognition (RER) sites located at the 5' end of the fragment,
wherein the RER sites are different from each other and, wherein at least one of

the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA upstream of the 5' end of the fragment;

two restriction enzyme recognition (RER) sites located at the 3' end of the fragment,

wherein the RER sites are different from each other and, wherein at least one of
the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the fragment;

isolating mRNA from said cell;

reverse transcribing isolated RNA into single stranded cDNA using a primer complementary to the sequence of the marker fragment;

extending the 3' end of the single stranded cDNA with a homopolymeric polydeoxynucleotide sequence using a single deoxynucleotide triphosphate and an enzyme terminal transferase;

synthesizing a second and complementary cDNA strand using a DNA polymerase and a primer complementary to the homopolymeric sequence;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes one of the Type IIS RER sites located at the 5' end of the marker fragment and thereupon cleaving the cDNA upstream of the 5' end of the marker fragment, thereby producing a cDNA fragment comprising the marker fragment and portions of upstream cellular tags;

ligating a linker to the cDNA fragment;

amplifying the ligation products with primers complementary to the marker fragment and to the ligated linker;

subjecting the amplification products to one or more restriction enzymes that recognize the RER ~~site~~site not previously recognized by the Type IIS restriction ~~enzymes~~enzyme used;
cloning the fragments;
obtaining the nucleotide sequence of the cloned fragments; and
comparing the individual sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 39 (Original): The method of claim 38 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claim 40 (Currently amended): A method for elucidating a RNA transcription profile in a cell comprising:

introducing into the cell a polynucleotide marker fragment, the expression of which is obtained

only upon integration of the polynucleotide ~~construct~~marker fragment into an actively transcribing genome region of the cell, wherein the polynucleotide marker fragment comprises in a 5' to 3' direction:

two restriction enzyme recognition (RER) sites located at the 5' end of the marker

fragment, wherein the RER sites are different from each other and, wherein at

least one of those RER sites is recognized by a Type IIS restriction enzyme, and

wherein this RER site is oriented so that a Type IIS restriction enzyme will cut the DNA upstream of the 5' end of the fragment;

two restriction enzyme recognition (RER) sites located at the 3' end of the marker

fragment, wherein the RER sites are different from each other and, wherein at

least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end of the marker fragment;

isolating mRNA from said cell;

reverse transcribing isolated RNA into single stranded cDNA;

synthesizing a second complementary strand of cDNA with a DNA polymerase and a primer whose sequence corresponds to the sequence of the marker fragment;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes one of the Type IIS RER sites located at the 3' end of the marker fragment and cleaving the cDNA downstream of the 3' end of the marker fragment, such that a cDNA fragment is produced comprising the marker, and portions of downstream cellular tags;

ligating a linker to the cDNA fragment;

amplifying the ligation products with primers corresponding to the marker and to the ligated linker;

subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction ~~enzymes~~enzyme used;

cloning the fragments;

obtaining the nucleotide sequence of the cloned fragments; and

comparing the individual sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 41 (Original): The method of claim 40 further comprising ligating the amplified fragments together to form a concatamer prior to cloning.

Claim 42 (Original): The method of claim 36, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 43 (Original): The method of claim 37, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 44 (Original): The method of claim 38, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 45 (Original): The method of claim 39, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 46 (Original): The method of claim 40, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 47 (Original): The method of claim 41, wherein the polynucleotide marker fragment is introduced into the cell by transfection.

Claim 48 (Original): A polynucleotide construct comprising in a 5' to 3' orientation:
a splice acceptor sequence;
a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving DNA
fused upstream of the 5' end of a marker exon;

a restriction enzyme recognition site;
a marker exon;
a restriction enzyme recognition site;
a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving
sequences located downstream of the 3' end of the marker exon; and
a splice donor sequence.

Claim 49 (Original): A vector comprising the polynucleotide construct of claim 48.

Claim 50 (Currently amended): The polynucleotide construct of claim 48 further
comprising a polynucleotide sequence encoding a ~~positive~~-selection marker located downstream
from the marker exon.

Claim 51 (Original): The polynucleotide construct of claim 48, wherein the marker exon
encodes a fluorescent protein marker.

Claim 52 (Original): The polynucleotide construct of claim 51, wherein the fluorescent
protein marker is green fluorescent protein.

Claim 53 (Original): The polynucleotide construct of claim 48, wherein the Type IIS
restriction enzyme recognition site is selected from the group consisting of BsmFI and MmeI.

Claim 54 (Original): The polynucleotide construct of claim 48, wherein the restriction enzyme recognition site is selected from the group consisting NcoI and BamHI.

Claim 55 (Original): A polynucleotide construct comprising in a 5' to 3' orientation:
a splice acceptor sequence;
a restriction enzyme recognition site;
a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving DNA
fused upstream of the 5' end of a marker exon;
a marker exon;
a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving sequences
located downstream of the 3' end of the marker exon;
a restriction enzyme recognition site;
a splice donor sequence.

Claim 56 (Original): A vector comprising the polynucleotide construct of claim 55.

Claim 57 (Currently amended): The polynucleotide construct of claim 55 further
comprising a polynucleotide sequence encoding a ~~positive~~-selection marker located downstream
from the marker exon.

Claim 58 (Original): The polynucleotide construct of claim 55, wherein the marker exon
encodes a fluorescent protein marker.

Claim 59 (Original): The polynucleotide construct of claim 58, wherein the fluorescent protein marker is green fluorescent protein.

Claim 60 (Original): The polynucleotide construct of claim 55, wherein the Type IIS restriction enzyme recognition site is selected from the group consisting of BsmFI and MmeI.

Claim 61 (Original): The polynucleotide construct of claim 55, wherein the restriction enzyme recognition site is selected from the group consisting NcoI and BamHI.

Claim 62 (Original): A polynucleotide construct comprising in a 5' to 3' orientation:
a splice acceptor sequence;
a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving DNA
fused upstream of the 5' end of a marker exon;
a restriction enzyme recognition site; and
a marker exon; and
a polyadenylation sequence.

Claim 63 (Original): A vector comprising the polynucleotide construct of claim 62.

Claim 64 (Original): The polynucleotide construct of claim 62, wherein the marker exon encodes a fluorescent protein marker.

Claim 65 (Original): The polynucleotide construct of claim 64, wherein the fluorescent protein marker is green fluorescent protein.

Claim 66 (Original): The polynucleotide construct of claim 62, wherein the Type IIS restriction enzyme recognition site is MmeI or BsmFI.

Claim 67 (Original): The polynucleotide construct of claim 62, wherein the restriction enzyme recognition site is NheI.

Claim 68 (Original): A polynucleotide construct comprising:
a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and
wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 5' end of the marker exon, wherein at least one the 5' RER sites is recognized by a Type IIS restriction enzyme and oriented in such a way that a Type IIS restriction enzyme cuts the DNA outside the boundaries that define the marker exon, and
wherein the marker exon contains at least two RER sites at the 3' end of the marker exon, wherein at least one of the 3' RER sites is recognized by a Type IIS restriction enzyme and oriented in such a way that a Type IIS restriction enzyme cuts the DNA outside the boundaries that define the marker exon, and
wherein said restriction recognition sites are located close from the border of the marker exon such that after cutting flanking exons generate sequence tags of at least 8 nucleotides.

Claim 69 (Original): A vector comprising the polynucleotide construct of claim 68.

Claim 70 (Original): A polynucleotide construct comprising:

a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and

wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 5' end of the marker exon, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and

wherein this Type IIS RER site is oriented so that a Type IIS restriction enzyme will cut DNA upstream of the 5' end the marker exon.

Claim 71 (Original): A vector comprising the polynucleotide construct of claim 70.

Claim 72 (Original): A polynucleotide construct comprising:

a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and

wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 3' end of the marker exon, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and

wherein this Type IIS RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end the marker exon.

Claim 73 (Original): A vector comprising the polynucleotide construct of claim 72.

Claims 74-75 (Cancelled).

Claim 76 (New). A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising in a 5' to 3' orientation:

- a splice acceptor sequence;

- a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving DNA fused upstream of the 5' end of a marker exon;

- a restriction enzyme recognition site;

- a marker exon;

- a restriction enzyme recognition site;

- a Type IIS restriction enzyme recognition site oriented so that it is capable of cleaving sequences located downstream of the 3' end of the marker exon; and

- a splice donor sequence;

isolating said RNA from said cell;

reverse transcribing the isolated mRNA into double stranded cDNA;

subjecting the cDNA to digestion with Type IIS restriction enzymes that recognizes the Type IIS

- RER sites located at the 5' and 3' ends of the marker exon and cleaving the cDNA

- upstream of the 5' end of the marker exon and downstream of the 3' end of the marker

- exon, thereby producing a cDNA fragment comprising the marker exon, and portions of

- upstream and downstream cellular exon tags;

self-ligating the cDNA fragment, thereby fusing the exon tags in opposing orientations;

amplifying a region of the cDNA fragment containing the exon tags in opposing orientations
thereby generating a linear DNA molecule containing the exon tags in opposing
orientations flanked by sequences corresponding to the marker exon 5' and 3' ends;
subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the
RER sites not previously recognized by the first Type IIS restriction enzymes, thereby
generating a linear DNA fragment containing upstream and downstream exon tags fused
in an inverted conformation;
cloning the fragments comprising the tags in inverted conformation;
obtaining the nucleotide sequence of the cloned tags; and
comparing the individual sequence tags or pairs of sequence tags to a sequence database such
that the RNA transcript corresponding to the sequenced tags is identified.

Claim 77 (New). A method for elucidating a RNA transcription profile in a eukaryotic cell
comprising:

introducing into a cell a polynucleotide construct comprising in a 5' to 3' orientation:

- a splice acceptor sequence;
- a restriction enzyme recognition site;
- a Type IIS restriction enzyme recognition site oriented so that it is capable of
cleaving DNA fused upstream of the 5' end of a marker exon;
- a marker exon;
- a Type IIS restriction enzyme recognition site oriented so that it is capable of
cleaving sequences located downstream of the 3' end of the marker exon;
- a restriction enzyme recognition site; and

a splice donor sequence;

isolating said RNA from said cell;

reverse transcribing the isolated mRNA into double stranded cDNA;

subjecting the cDNA to digestion with Type IIS restriction enzymes that recognizes the Type IIS RER sites located at the 5' and 3' ends of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon and downstream of the 3' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon, and portions of upstream and downstream cellular exon tags;

self-ligating the cDNA fragment, thereby fusing the exon tags in opposing orientations;

amplifying a region of the cDNA fragment containing the exon tags in opposing orientations thereby generating a linear DNA molecule containing the exon tags in opposing orientations flanked by sequences corresponding to the marker exon 5' and 3' ends;

subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the RER sites not previously recognized by the first Type IIS restriction enzymes, thereby generating a linear DNA fragment containing upstream and downstream exon tags fused in an inverted conformation;

cloning the fragments comprising the tags in inverted conformation;

obtaining the nucleotide sequence of the cloned tags; and

comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 78 (New). A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising in a 5' to 3' orientation:

a splice acceptor sequence;

a Type IIS restriction enzyme recognition site oriented so that it is capable of

cleaving DNA fused upstream of the 5' end of a marker exon;

a restriction enzyme recognition site;

a marker exon; and

a polyadenylation sequence;

isolating said RNA from said cell;

reverse transcribing the isolated mRNA into single stranded cDNA using a primer of sequence

complementary to the sequence of the marker exon;

extending the 3' end of the single stranded cDNA with a homopolymeric polydeoxynucleotide

sequence using a single deoxynucleotide triphosphate and an enzyme terminal

transferase;

synthesizing a second and complementary cDNA using a DNA polymerase and a primer

complementary to the homopolymeric sequence;

subjecting the cDNA to digestion with at a Type IIS restriction enzyme that recognizes a Type

IIS RER site located at the 5' end of the marker exon and cleaving the cDNA upstream of

the 5' end of the marker exon, thereby producing a cDNA fragment comprising the

marker exon, and portions of upstream cellular exon tags;

ligating a linker to the cDNA fragment;

amplifying the linker and cDNA fragment with primers complementary to the marker exon and

to the ligated linker;

subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction enzyme;
cloning the fragments comprising the tags in inverted conformation;
obtaining the nucleotide sequence of the cloned tags; and
comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 79 (New). A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising:

a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 5' end of the marker exon, wherein at least one the 5' RER sites is recognized by a Type IIS restriction enzyme and oriented in such a way that a Type IIS restriction enzyme cuts the DNA outside the boundaries that define the marker exon, and wherein the marker exon contains at least two RER sites at the 3' end of the marker exon, wherein at least one of the 3' RER sites is recognized by a Type IIS restriction enzyme and oriented in such a way that a Type IIS restriction enzyme cuts the DNA outside the boundaries that define the marker exon, and wherein said restriction recognition sites are located close from the border of the marker exon such that after cutting flanking exons generate sequence tags of at least 8 nucleotides;

isolating said RNA from said cell;

reverse transcribing the isolated mRNA into double stranded cDNA;
subjecting the cDNA to digestion with Type IIS restriction enzyme that recognize one Type IIS RER site located at the 5' end of the marker and one Type IIS RER site located at the 3' end of the marker exon and cleaving the cDNA upstream of the 5' end of the marker exon and downstream of the 3' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon, and portions of upstream and downstream cellular exon tags;
self-ligating the cDNA fragment, thereby fusing the exon tags in opposing orientations;
amplifying a region of the cDNA fragment containing the exon tags in opposing orientations thereby generating a linear DNA molecule containing the exon tags in opposing orientations flanked by sequences corresponding to the marker exon 5' and 3' ends;
subjecting the amplified cDNA fragment to one or more restriction enzymes that recognize the RER sites not previously recognized by the first Type IIS restriction enzymes, thereby generating a linear DNA fragment containing upstream and downstream exon tags fused in an inverted conformation;
cloning the fragments comprising the tags in inverted conformation;
obtaining the nucleotide sequence of the cloned tags; and
comparing the individual sequence tags or pairs of sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.

Claim 80 (New). A method for elucidating a RNA transcription profile in a eukaryotic cell comprising:

introducing into a cell a polynucleotide construct comprising:

a marker exon sequence flanked by a functional 5' splice acceptor sequence and a 3' splice donor sequence, and wherein said marker exon contains at least two restriction enzyme recognition (RER) sites at the 3' end of the marker exon, wherein at least one of the RER sites is recognized by a Type IIS restriction enzyme, and wherein this Type IIS RER site is oriented so that a Type IIS restriction enzyme will cut DNA downstream of the 3' end the marker exon;

isolating said RNA from said cell;

reverse transcribing the isolated mRNA into single stranded cDNA;

synthesizing a second complementary strand of cDNA with a DNA polymerase enzyme and a primer whose sequence corresponds to the sequence of the marker exon;

subjecting the cDNA to a Type IIS restriction enzyme that recognizes one Type IIS RER site located at the 3' end of the marker exon and thereupon cleaves the cDNA downstream of the 3' end of the marker exon, thereby producing a cDNA fragment comprising the marker exon and portions of downstream cellular exon tags;

ligating a linker to the cDNA fragment comprising the marker exon fused to a downstream flanking cellular exon tag;

amplifying the cellular exon tag with primers complementary to the marker exon and to the ligated linker;

subjecting the amplification products to one or more restriction enzymes that recognize the RER sites not previously recognized by the Type IIS restriction enzyme used;

cloning the fragments;

obtaining the nucleotide sequence of the cloned tags; and

comparing the individual sequence tags to a sequence database such that the RNA transcript corresponding to the sequenced tags is identified.